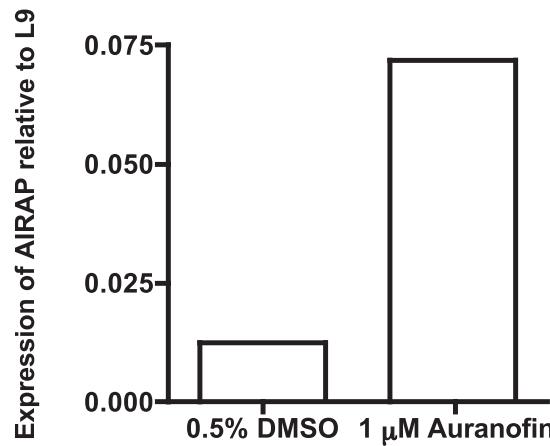
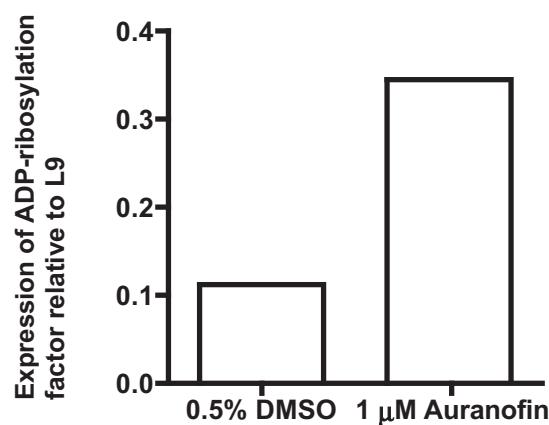
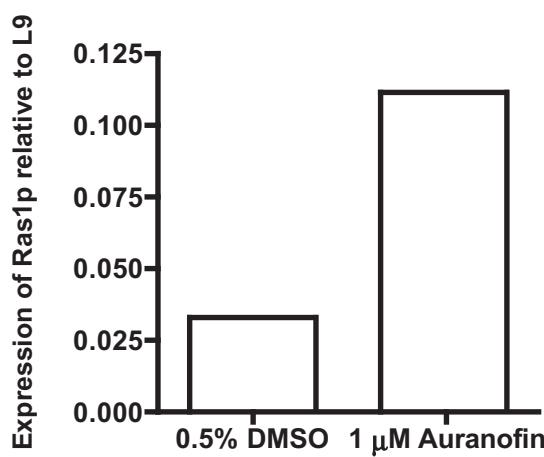
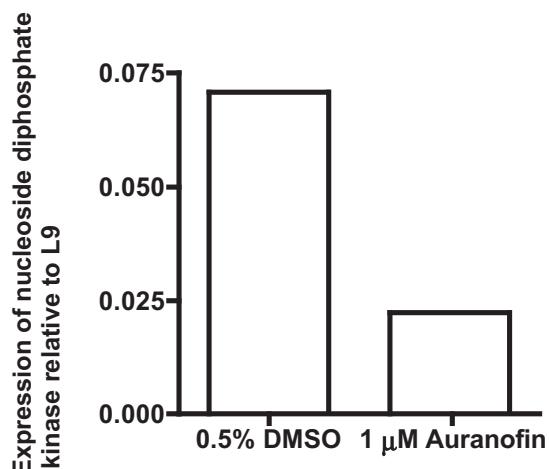
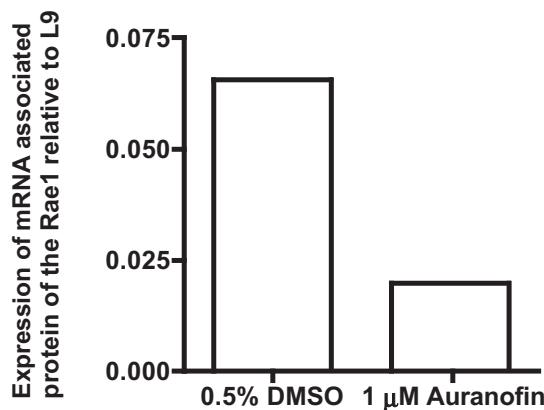


## **SUPPLEMENTARY INFORMATION**

### **A high throughput drug screen for *Entamoeba histolytica* identifies a new lead and target**

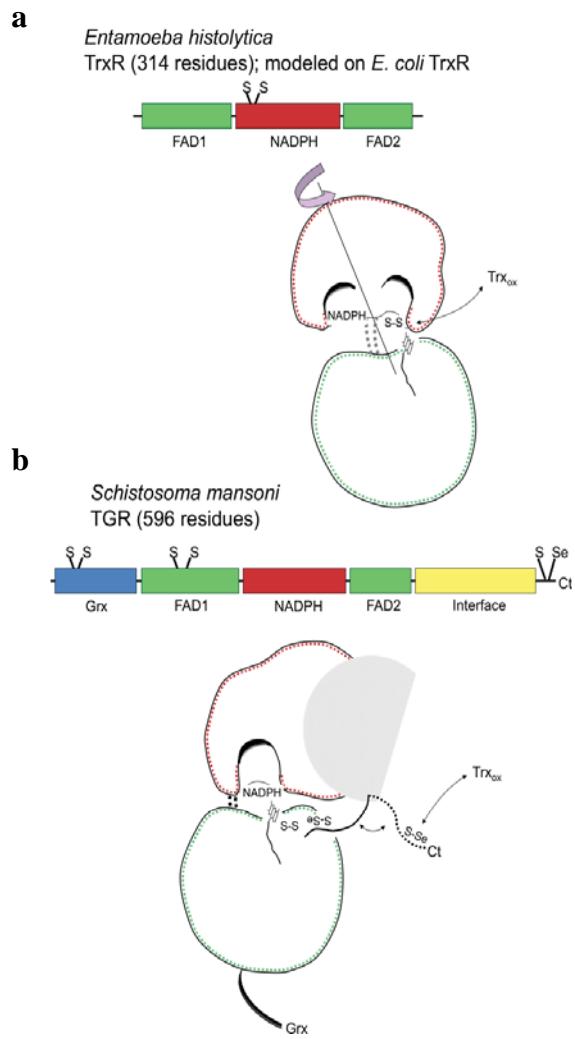
Anjan Debnath, Derek Parsonage, Rosa M Andrade, Chen He, Eduardo R Cobo, Ken Hirata, Steven Chen, Guillermina García-Rivera, Esther Orozco, Máximo B Martínez, Shamila S Gunatilleke, Amy M Barrios, Michelle R Arkin, Leslie B Poole, James H McKerrow & Sharon L Reed



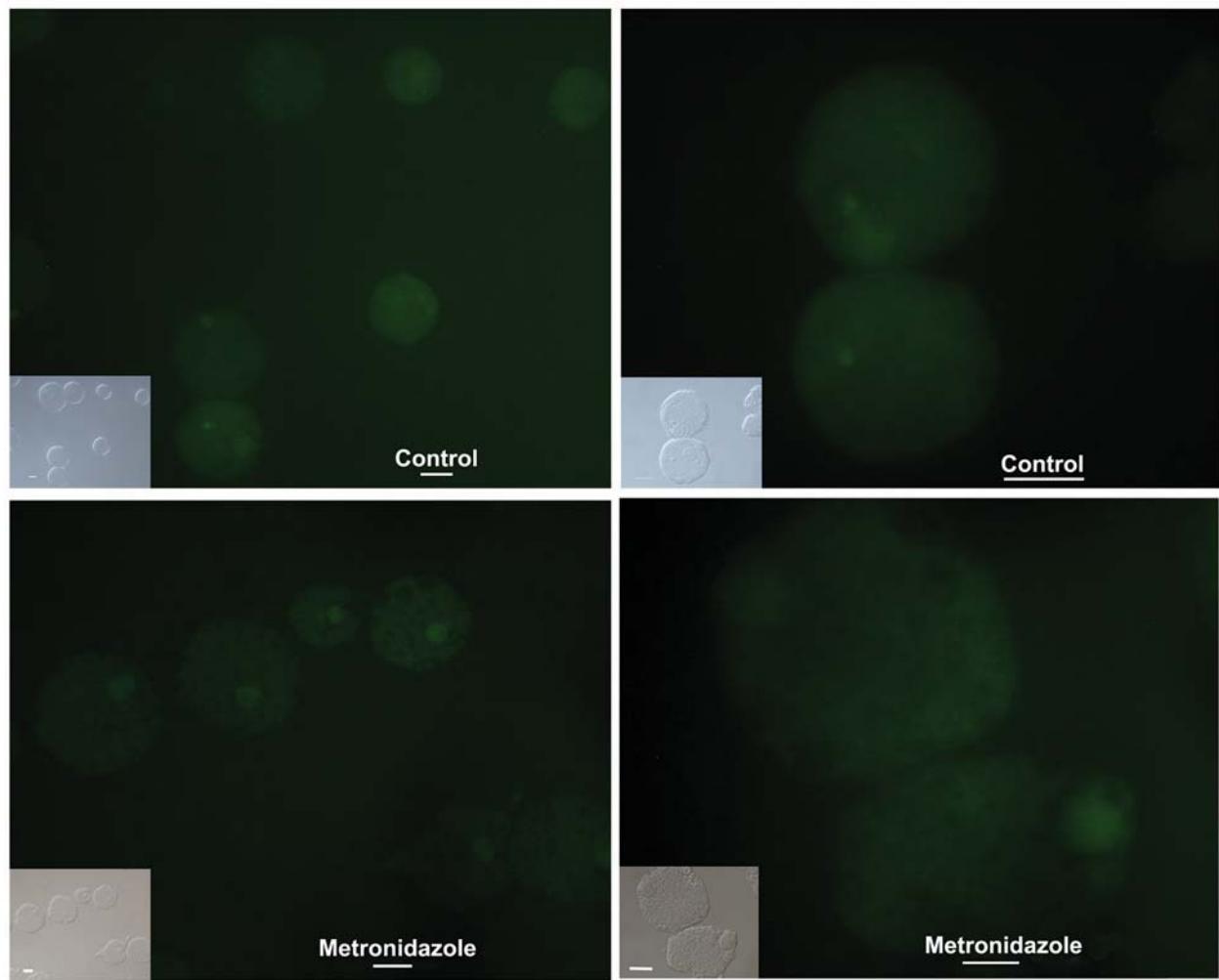
**Supplementary Figure 1.** Verification of gene expression by qRT-PCR analysis. Quantitative values for qRT-PCR were obtained from the threshold cycle ( $C_T$ ) number at which the increase in the signal associated with an exponential growth of PCR products was first detected. Final results were obtained by comparative  $C_T$  method using average  $C_T$  values of two independent biological samples and normalized to the value of a housekeeping gene (*E. histolytica* L9 ribosomal protein) and expressed as the expression of gene of interest relative to the reference gene *E. histolytica* L9 ribosomal protein. The genes selected for confirmation by qRT-PCR analysis are mRNA associated protein of the Rae1, nucleoside diphosphate kinase, Ras1p, ADP-ribosylation factor, AIRAP.

EhTrxR	-----	
EcTrxR	-----	M 1
SmTGR	MPPADGTSQWLRTKTVDSAAILFSKTTCPYCKVKDVLAEEKIKHATIELDQLSNGSAIQ	60
EhTrxR	-----MSNIHDVVIIGSGP---	14
EcTrxR	TCLSLMLPIAQIVNKIVILFFYVCKFPTILPLSANN--YGDLMGTTKHSKLILGSGP---	56
SmTGR	KCLASFSKIEVTVPQMFRGKFIGDSQTVLKYYSNDELAGIVNESKYDYDLIVIGGGSGGL	120
	.....::.*..	
EhTrxR	-AAHTAAIYLGRSSLKPVMYEGFMAGGVAAAGG-----	45
EcTrxR	-AGYTTAAVVAARANLQPVLIT-----GMEKGG-----	82
SmTGR	AAGKEAAKYGAKTAVLDYVEPTPIGTTWGLGGTCVNVCIPKKLMHQAGLLSHALEDAEH	180
	*. ** * . : : :	
EhTrxR	--QLTTTTIIIFIENFPFPNGIDGNELMMNNMRTQSEKYGTIIITETIDHVDFSTQPFKLFT	102
EcTrxR	--QLTTTTEVENWPGPNDLTGPLLTERMHEATKFETEIIIFDHINKVQLQNRPFRLTG	139
SmTGR	FGWSLDRSKISHNWSTMVEGVQSHIGSLNWGYKVALRDNQVTLYNAKGRLISPHEVQITD	240
	. * .. .*:.. .: . : . : . : . : . : . : .	
EhTrxR	EEGKEVLTKS--VIIATGATAKRHMVPGEDKYWQNQVSACACIDGAVPIFRNKVLMVVGG	160
EcTrxR	DSG-EYTCD--LIIATGASARYLGLPSEEAFKGRGV SACATCDG--FFYRNQKVAVIGG	194
SmTGR	KNQKVSTITGNKIIILATGERPKYPEIPGAVEY--GITSDDLFLSP--YFPGKTLVIGA	294
	... . : * : *** .. : * . : * : : . : * : * : .	
EhTrxR	GDAAMEEALHLTKYGSKVIILHRRDAFR-ASKTMQERVLNHP---KIEVIWN-----SE	210
EcTrxR	GNTAVEEALYLSNIASEVHLLIHRRDGFR-AEKILIKRIMDKVENGNIILHTN-----RT	247
SmTGR	SYVALECAGFLASLGGDVTVVMVRSSLRGFDQQMAEKVGDYMENHGVKFAKLCVPDEIKQ	354
	. . * : * . * . . . * : * . : * . : . : . : .	
EhTrxR	LVELEGDGDLLNGAKIHN-LVSGEYKVVPVAGLFYAIIGHSP---NSKFLGGQVKTADDGY	266
EcTrxR	LEEVITGDQMVGVTGVRLLRTDQNSDNIESLDVAGLFVAIIGHSP---NTAIFEGQLELEN-GY	303
SmTGR	LKVVDTENNKPGLLVLKGHYTDGKKFEEEFETVIFAVGREPQLSKVLCETVGVKLKDKNR	414
	* : : . : . : . : . : . : * : * : . : . : . : .	
EhTrxR	ILTEG-----PKTSVDGVFACGDVCDRVYRQAIVAAGSG-----	300
EcTrxR	IKVQSGIHGNATQTSIPGVFAAGDVMHDHYRQAITTSAGTG-----	343
SmTGR	VVCTDD-----EQITTVSNVVAIGDINAGKPQLTPVAIQAGRYLARRLFAGATELTDYSNV	469
	: . : * : . * : * : * : . : . : . : . : .	
EhTrxR	-----CMAALSCEKWLQTH-----	314
EcTrxR	-----CMAALDAERYLDGLADAK-----	361
SmTGR	ATTVFPTPLEYGACGLSEEDAEIKEYGDKDIEVYHSNFKPLEWTVAHREDNVCYMKLVCRK	529
	. . * . : .	
EhTrxR	-----	
EcTrxR	-----	
SmTGR	DNMRVLGLHVLPNAGEITQGYAVAIAKMGATKAADFRTIGIHPCTSETFTTLHVTKKSGV	589

**Supplementary Figure 2.** Comparison of deduced amino acid sequences of *E. histolytica* (EhTrxR), *E. coli* (EcTrxR) and *S. mansoni* (SmTGR) thioredoxin reductase genes aligned with Clustal W. The redox-active disulfide (motif CXXC) of TrxR of *E. coli* and *E. histolytica* and the redox-active disulfide motif which binds selenium (CXXXC) of SmTGR are underlined. Identical residues are marked with a “\*”, conserved substitutions with “:” and semi-conserved with “.”.

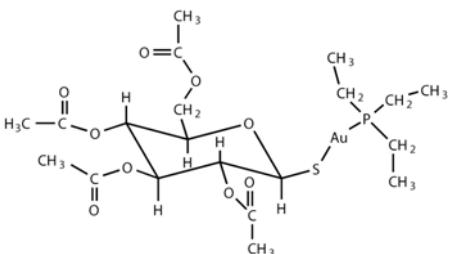
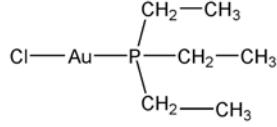
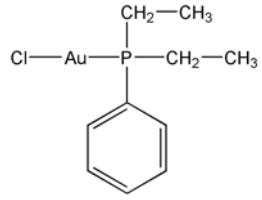
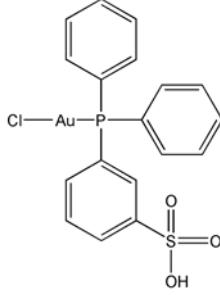


**Supplementary Figure 3.** Structural basis for catalysis by TrxRs. **(a)** Low molecular weight (mw) TrxRs are expressed by bacteria and some eukaryotes (e.g., *E. coli* and *E. histolytica*) and possess both the NADPH binding site and the redox-active disulfide (depicted as S-S) in the same domain (maroon, upper domain; one monomer of the dimeric protein is depicted for *E. coli* TrxR, PDB code 1CL0). By 67° rotation of the top domain, about the axis shown relative to the lower, FAD-binding domain (green, composed of two non-contiguous segments of the sequence), the disulfide/dithiol center is alternately located proximal to the isoalloxazine ring of the FAD (triple ring structure), or swung out for electron transfer to the oxidized thioredoxin (Trx<sub>ox</sub>) substrate<sup>41</sup>. **(b)** Higher mw TrxRs, expressed in mammalian cells as well as *Caenorhabditis elegans* and *Plasmodium*, are structurally related to glutathione reductases<sup>42</sup>. The closely related enzyme expressed in the blood fluke *Schistosoma mansoni* (depicted), includes an extra glutaredoxin (Grx) domain and exhibits both glutathione reductase and TrxR activities (a thioredoxin glutathione reductase, or TGR). The large TrxR and TGR proteins possess an additional redox center within the mobile C-terminal tail that alternately visits the disulfide/dithiol center of the other subunit within the dimer (gray domain), located on the opposite side of the FAD isoalloxazine ring from the NADPH binding site, or swings out to reduce Trx<sub>ox</sub>. In mammalian TrxR and *S. mansoni* TGR, this redox center is composed of a cysteine-selenocysteine sequence (S-Se); the selenocysteine plays an important role in the inhibition of these enzymes by auranofin<sup>23</sup>.



**Supplementary Figure 4.** Reactive oxygen species detection following treatment with metronidazole. Reactive oxygen species were not detected within trophozoites following treatment with 2  $\mu$ M metronidazole by fluorescence of dichlorofluorescein. Control trophozoites were treated with sterile water only. Scale bars 10  $\mu$ m.

**Supplementary Table 1.** *In vitro* inhibitory effects of auranofin and its analogs

Structure	EC <sub>50</sub> (μM)
 <b>Auranofin</b>	0.5
 <b>Analog 39</b>	0.5
 <b>Analog 7</b>	1
 <b>Analog 12</b>	5

**Supplementary Table 2.** Summary of the auranofin-responsive gene expression data.

Genes showing differential expression due to auranofin treatment were selected after scanning the microarrays. 1  $\mu$ M auranofin-treated *E. histolytica* amplified RNA was labeled with Cy5 and 0.5% DMSO-treated *E. histolytica* amplified RNA was labeled with Cy3. F635 is the fluorescence at 635 nm for Cy5 channel and F532 is the fluorescence at 532 nm for Cy3 channel. B635 and B532 are background values at 635 nm and 532 nm, respectively. The numerical values for the signal intensities were averaged from each oligonucleotide printed in triplicate and from two biological replicates.

Gene Name	Average F635 Median - B635	Average F532 Median - B532	Average Log <sub>2</sub> Ratio (635/532)
mRNA associated protein of the Rae1	334.33	2142.83	-3.03
Nucleoside diphosphate kinase	1610.33	19967.83	-3.36
Ras1p, putative	14784.83	4461.5	1.79
Similar to Arsenite-inducible RNA-associated protein	9809.33	1709.5	2.45
ADP-ribosylation factor	7171.17	1270.5	2.37

**Supplementary Table 3.** Oligonucleotide primers for qRT-PCR

Gene	Description	Sequence (5' to 3')
mRNA associated protein of the Rae1	qRT-PCR forward qRT-PCR reverse	CTGCTGGTAGTGATGGTGTGA CCTTGGTGCCAATCATATCC
Nucleoside diphosphate kinase	qRT-PCR forward qRT-PCR reverse	CCAACTGAATTGGCAGAACAA TGCCTTTCAACAAAAGTTGG
Ras1p, putative	qRT-PCR forward qRT-PCR reverse	GGGGTCATTGTTAATGCTCA GAAGGCACATGGACAAACCT
ADP-ribosylation factor	qRT-PCR forward qRT-PCR reverse	TGCCAATAAACATGACCTTCC TGATGAAAGCCAATCAAGACC
Similar to Arsenite-inducible RNA-associated protein	qRT-PCR forward qRT-PCR reverse	CCAATCAACCCATCATTGTG GCCTTCTTTGGGCACTTAAT
L9 ribosomal protein	qRT-PCR forward qRT-PCR reverse	CTTGTGTAAGAAGGAAGGAC AACAGCTGAATCTCTTCTATTTC

## **Supplementary References**

41. Lennon, B.W., Williams, C.H., Jr. & Ludwig, M.L. Twists in catalysis: alternating conformations of *Escherichia coli* thioredoxin reductase. *Science* **289**, 1190–1194, (2000).
42. Rahlfs, S., Schirmer, R.H. & Becker, K. The thioredoxin system of *Plasmodium falciparum* and other parasites. *Cell Mol. Life Sci.* **59**, 1024–1041 (2002).